syngenta

Chlorothalonil

A Source to Outcome Approach for Inhalation Risk Assessment

Final Report

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VOLUME 1 OF 1 OF STUDY

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Since this volume contains an assessment of data and is not a study per se, a Good Laboratory Practice (GLP) statement, as defined by 40 CFR Part 160, is not appropriate.

There is no GLP study director for this volume.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1.0 EXECUTIVE SUMMARY

The Source to Outcome Approach provides a framework for improving and modernizing human health risk assessments where uncertainty is reduced through integrating hazard and exposure characterization. For pesticides, such as chlorothalonil, using human-relevant particle size distributions (PSDs) from agricultural pesticide handling scenarios with refined inhalation dosimetry models, such as computational fluid dynamic (CFD) models, more accurately estimates a human equivalent concentration (HEC) for human health risk assessments.

Chlorothalonil (2,4,5,6-tetrachloroisophthalonitrile) is a broad spectrum, non-systemic pesticide mainly used as a fungicide to control fungal foliar diseases on a wide variety of vegetable, field and ornamental crops. It also has non-agricultural uses such as a wood protectant, anti-mold and anti-mildew agent, bactericide, microbiocide, algaecide and insecticide. Residential uses include golf courses, wood preservatives and use in paint formulations.

While exposure to chlorothalonil can occur for mixer/loaders, applicators, and bystanders, only exposure to spray applicators is discussed herein as an example of the Source to Outcome approach. Pesticide spray applicator inhalation exposure to chlorothalonil is mainly in the form of aerosols or particulates. When exposure is sufficiently high, it can result in toxicological effects. Inhalation exposure data used by USEPA in human health risk assessments for operators/handlers are typically measured with personal air monitors (e.g. OSHA Versatile Sampler (OVS) tubes). The PSD of the particles captured by these devices is critical information that can be used in dosimetry models in order to derive precise estimates of inhalation exposure. Side-by-side air sampling with OVS tubes and Respicon TM particle samplers was conducted to characterize the size distribution of aerosols collected on OVS tubes. The study results showed that the OVS tubes sample the inhalable fraction (<100 μm). Using well established sampling distribution conventions, a representative PSD (Mass Median Aerodynamic Diameter or MMAD = 35 μm and Geometric Standard Deviation or GSD = 1.5) was mathematically defined for spray applicators with a cut-off of 100 μm.

Dosimetry provides an important link to understanding the relationship between external exposure and biological response. The use of appropriate dosimetry tools improves the accuracy of risk assessments, and reduces the uncertainty of the internal dose estimates at target tissues used to derive the HEC or human equivalent dose (HED) values. A three-dimensional CFD model (Corley, et al., 2015) (Corley, et al., 2012) (Kabilan, et al., 2016)) was used to calculate the surface concentrations of deposited chlorothalonil spray aerosol within the respiratory tract of typical applicators.

For respiratory irritants, such as chlorothalonil, an alternative *in vitro* approach may be taken when *in vivo* data are not adequate or not available to establish a toxicity endpoint for inhalation risk assessment. An *in vivo* study would only show the toxicological effects at the target tissues in the respiratory tract, but not the target tissue exposure concentration that caused the effect. The MucilAir™ system was identified as the optimal *in vitro* model to

assess damage to respiratory epithelial cells caused by exposure to chlorothalonil. Using three different endpoints that measured the integrity of tight junctions between cells in the membrane, cytotoxicity, and cell metabolic competence, a Benchmark Dose Level (BMDL) of 0.00730 mg/cm^2 was derived for chlorothalonil.

Using the human-relevant inhalation PSD (MMAD = 35 µm; GSD = 1.5), human dosimetry modelling using the CFD model, and the MucilAir™ BMDL, an HEC for the chlorothalonil applicator risk assessment was derived for various sub-regions in the respiratory tract. The lowest HEC was for the larynx, 0.037 mg/L, and was used in the spray applicator risk assessment for chlorothalonil. Based on the guidance provided by the USEPA on data-derived extrapolation factors (DDEF) (USEPA, 2014), the traditional 10X interspecies can be reduced to 1X due to the use of an endpoint derived from human respiratory tissue and human dosimetry results from the CFD model. Therefore, using the proposed approach would result in a net LOC of 10 (10X intraspecies and 1X interspecies) for operator inhalation risk assessments. The Margins of Exposure (MOE) calculated for several representative spray application scenarios show that exposure levels are within acceptable limits, resulting in reasonable certainty of no harm to the applicators.

The Source to Outcome Approach yielded more precise and accurate respiratory dose estimates for liquid spray applicators, resulting in chlorothalonil risk assessments that are precise, accurate and health protective. This same approach could also be used for other operator scenarios for chlorothalonil, such as mixing/loading, by integrating appropriate PSDs and assumptions for % chlorothalonil relevant for these scenarios. This approach resulted in several important findings pertaining to chlorothalonil inhalation risk assessment:

1. OVS tubes sample the inhalable fraction (<100 µm), with PSD of aerosols identified relevant for human exposures.
2. The *in silico* (CFD) model yielded dosimetry estimates for the human upper respiratory tract in the critical sub-regions of interest (e.g., larynx).
3. The *in vitro* (MucilAir^{TM}) study established a human BMDL (0.00730 mg/cm^2).
4. The elements of the source to outcome approach allow the calculation of DDEFs in place of standard uncertainty factors.
5. The HEC extrapolated from the *in vitro* BMDL resulted in risk assessments for spray applicators that were not of concern (i.e., MOEs> LOC of 10).

2.0 INTRODUCTION

Chlorothalonil (CTN), IUPAC name tetrachloroisophthalonitrile, is a non-systemic broad spectrum multisite fungicide used to control fungal foliar diseases on a wide variety of field and greenhouse crops, and plays an important role in preventing fungicide resistance. Inhalation exposure to CTN may occur during mixing/loading or applying CTN containing products. Exposure is typically measured using air sampling devices, such as OVS tubes, which capture aerosols or particles on a filter and sorbent, and an inhaled dose or concentration is calculated. This exposure concentration is compared with the HEC extrapolated from a point of departure or effect level, such as the no observed adverse effect level (NOAEL), when conducting inhalation risk assessments.

Currently, the standard approach taken by USEPA for conducting inhalation risk assessment for pesticides, such as CTN, involves calculating an HEC extrapolated from a rat inhalation toxicity point of departure (POD), using the regional deposited-dose (RDD) model (USEPA, 1994). The RDDs can be calculated in the human and rat respiratory tract for the extrathoracic, tracheobronchial, pulmonary or total respiratory tract depending on the region relevant to the adverse health outcome. The calculated RDD ratio (RDDR) is used to adjust the rat deposited dose to the HEC for use in risk assessment. While many risk assessments are based on POD adjustments derived using this method, the RDD model has several limitations for estimating deposition in human airways. For example, the RDD model is not able to incorporate relevant PSDs which would be inhaled by a human, provide estimation of particle clearance or retention, nor is it able to estimate deposition in discrete regions of the upper respiratory tract. Additionally, use of the RDDR software has decreased over time with development of alternative software tools such as the multiple path particle dosimetry (MPPD) model for humans and rats (Kuempel, Sweeney, Morris, & Jarabek, 2015). The MPPD model includes respiratory tract models of the deposition and clearance of particles and allows PSDs as input parameters for both the human and rat. Therefore, when comparing regional deposition in both rat and human, the MPPD model is an optimal default (Tier 1) screening tool. CFD models provide an additional level of refinement by estimating deposition in discrete regions of the upper respiratory tract. Therefore, under some circumstances where inhalation risk assessments exceed the level of concern (LOC), refinement is warranted and an alternative dosimetry tool may be necessary. This paper describes a refined inhalation risk assessment approach that considers the PSD generated during spray application of crop protection products and dosimetry in site-specific regions of the upper respiratory tract using CFD modelling.

3.0 SOURCE TO OUTCOME APPROACH AND CONCEPTUAL MODEL

3.1 Source to Outcome Approach

The Source to Outcome Approach (Figure 1) provides a framework for integrating exposure and hazard characterization to refine inhalation risk assessment of irritant aerosols. Components of the approach can be tailored to address specific questions related to inhalation exposure and risk for pesticide actives. This paper describes each component of the approach (Source, Exposure, Dosimetry and Outcome) and the application of the approach in refining CTN inhalation risk assessment for operators applying liquid formulations.

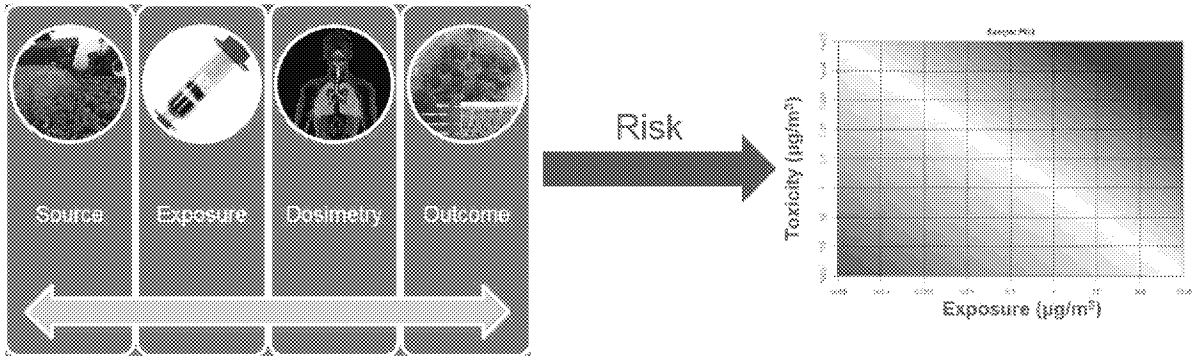


Figure 1 Source to Outcome Approach for Inhalation Risk Assessment

3.2 Conceptual Model

The application of the Source to Outcome Approach for CTN inhalation risk assessment can be described using the following conceptual model (Figure 2). Particle size characterization from pesticide use is integrated with *in silico* inhalation dosimetry modeling in human airways using CFD models and compared with the MucilAir™ *in vitro* BMDL to derive a HEC. For the risk assessment, the HEC is directly compared with CTN inhalation exposure for different operator exposure scenarios applying liquid formulations.

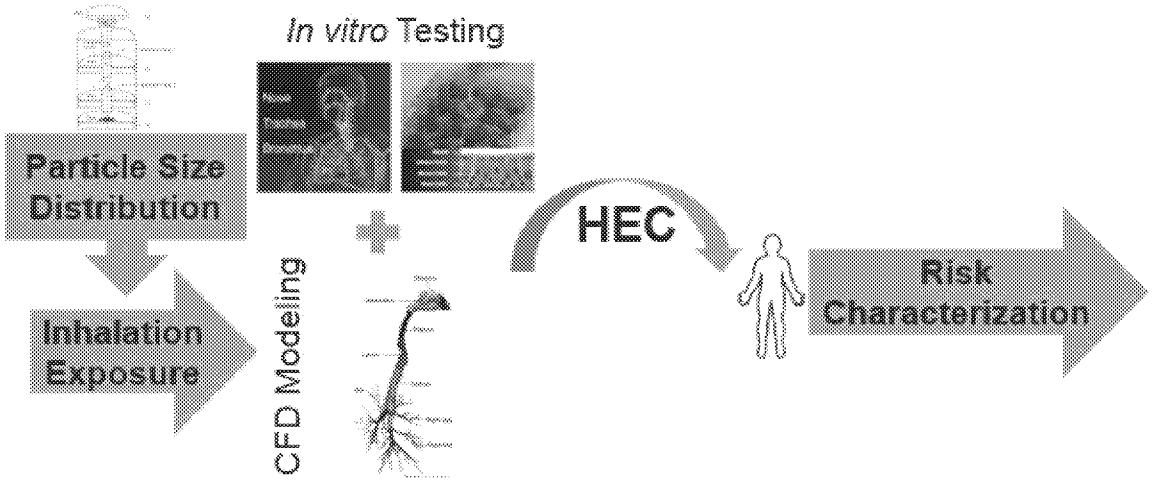


Figure 2 Conceptual Model for CTN inhalation risk characterization

3.3 Organization of this Document

This document is divided into Chapters, which will guide the reader through each particular area of the Source to Outcome Approach. The document starts with a description of CTN use patterns and droplet size distribution of nozzle sprays (Source) and concludes with integration of the approach resulting in the Operator Inhalation Risk Assessment for CTN spray applicators. These Chapters are briefly described as follows:

- **Source** (Chapter 4.0): This chapter describes Syngenta CTN solo and mixed use products applied as liquids registered by USEPA and CTN use patterns.

- **Exposure** (Chapter 5.0): This chapter describes characterization of the inhaled aerosols collected on personal air monitoring samplers (OVS tubes) during spray applications. A representative PSD was defined for spray applicators using internationally accepted sampling criteria.

- **Dosimetry** (Chapter 6.0): This chapter describes dosimetry calculations for the human upper respiratory tract using CFD modelling. Deposition was calculated for the PSDs identified for applicators using liquid formulations.

- **Outcome** (Chapter 7.0): This chapter summarizes the *in vitro* MucilAir^{TM} model of the human respiratory tract and calculation of a toxicological endpoint or BMDL for chlorothalonil.

- **Operator Risk Assessments** (Chapter 8.0): This chapter describes calculation of the HEC using CFD dosimetry values and endpoint (BMDL) values. As an example of using this approach, this chapter evaluates risk assessment for spray applicators.

4.0 SOURCE

The application methods and product formulations used by pesticide handlers are important considerations when evaluating inhalation exposure to pesticide active ingredients. This chapter summarizes the occupational use patterns and physical-chemical properties of chlorothalonil (CTN), as well as the range of nozzle spray qualities resulting from CTN use. This information was used to better understand the relationship between nozzle spray quality and potential inhalation exposure, and not used directly in deriving the PSD used in the risk assessment. For the purposes of this paper, standard PSDs were used (as presented in Section 5). This chapter uses the terms droplet size distribution (DSD), to refer to water-only droplets (i.e., not containing CTN), and PSD, to refer to aerosols containing CTN.

4.1 Uses and Physical-Chemical Properties of Chlorothalonil

Chlorothalonil (2,4,5,6-tetrachloroisophthalonitrile) is a broad spectrum, non-systemic pesticide mainly used as a fungicide to control fungal foliar diseases on a wide variety of vegetable, field and ornamental crops (Figure 3). It is also used as a wood protectant, anti-mold and anti-mildew agent, bactericide, microbiocide, algaecide and insecticide. Residential uses include golf courses, wood preservatives and use in paint formulations. CTN can be applied using a variety of application methods, including hand-held equipment, groundboom, chemigation, airblast and aerial applications. The main objective of this work, which is described in the following chapters, is to characterize and evaluate exposure and risk to CTN liquid formulations or solids that are ultimately diluted and applied as liquid. These CTN products include suspension concentrates (SC), suspo-emulsions (SE) or water dispersible granules (WDG). A summary of Syngenta CTN products applied as a liquid are summarized in Table 1. The highest percent CTN formulated as a liquid is 54% w/w, and highest percent CTN in diluted tank mix applied as a liquid is 4.9%. The precent of active ingredient in the formulated product will vary slightly between batches during production.

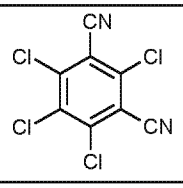
Chlorothalonil	

Figure 3 Chemical structure of Chlorothalonil

Table 1 **Summary of Syngenta Chlorothalonil products registered by USEPA applied as a liquid spray**

Product (Reg number)	Formulation Type[1]	% CTN (w/w) in product	Max Label Rate (lb ai/A)	Diluted Spray Conc. (lb ai/gal)[2]	% CTN (w/v) in Diluted Spray
Liquid Formulations					
Chlorothalonil Flowable 720 (100-1394)	SC	54%	11.3	0.38	4.5%
Daconil Action (100-1364)	SC	54%	11.3	0.38	4.5%
Daconil 720 Flowable Fungcide (50534-209)	SC	54%	11.3	0.38	4.5%
Daconil ZN Flowable Fungicide (50534-211)	SC	38.50%	11.3	0.38	4.5%
Instrata (100-1231)	SE	29.90%	11.2	0.37	4.5%
Bravo 500 (50534-8)[5]	SC	40.40%	5.0	0.25	3.0%
Tilt Bravo SE (100-1192)	SE	38.50%	1.1	0.22	2.6%
Quadris Opti (100-1171)	SC	46%	2.5	0.17	2.0%
Renown Fungicide (100-1315)	SC	45%	7.3	0.15	1.7%
Revus Opti (100-1279)	SC	33.30%	1.3	0.13	1.6%
Bravo 720 (50534-188)[3]	SC	54%	11.2	0.12	1.4%
Bravo Top (100-1441)	SC	40%	1.15	0.12	1.4%
Bravo ZN (50534-204)[4]	SC	38.50%	11.4	0.092	1.1%
Ridomil Gold Bravo SC (100-1221)	SC	33.10%	1.4	0.093	1.1%
Orondis Opti (100-1591)	SC	33.20%	1.0	0.067	0.8%
Concert II (100-1347)	SE	38.50%	11.3	0.022	0.3%

Product (Reg number)	Formulation Type[1]	% CTN (w/w) in product	Max Label Rate (lb ai/A)	Diluted Spray Conc. (lb ai/gal)[2]	% CTN (w/v) in Diluted Spray
Solid Formulations Applied as Liquid					
Chlorothalonil 82.5 SDG (100-1395)	WDG	82.50%	4.1	0.41	4.9%
Daconil SDG (50534-202)	WDG	82.50%	4.6	0.02	0.3%
Bravo 825 Agricultural Fungicide (50534-201)[6]	WDG	82.50%	11.2	0.021	0.2%

[1] SC = suspension concentrate, SE = suspo-emulsion, WDG = water dispersible granule

[2] Diluted spray tank concentration based on label directions for use.

[3] Registration transferred to ADAMA on September 18, 2017. ADAMA USEPA Registration No. 66222-276.

[4] Registration transferred to ADAMA on September 18, 2017. ADAMA USEPA Registration No. 66222-278.

[5] Registration transferred to ADAMA on September 18, 2017. ADAMA USEPA Registration No. 66222-275.

[6] Registration transferred to ADAMA on September 18, 2017. ADAMA USEPA Registration No. 66222-277.

5.0 EXPOSURE

For operators/handlers, inhalation exposure to CTN is mainly in the form of aerosols or particulates. When exposure is sufficiently high, it can result in toxicological effects. The PSD of agricultural sprays depends on the nozzle type, operating pressure, and the physical properties of the tank-mixture, which together determine the spray quality (e.g., fine, medium, coarse). Often in the analysis of spray PSD, interactions among formulation, nozzle types and operating pressures are observed. Therefore, the relationship between nozzle type or spray quality (e.g., fine, medium, coarse) and potential inhalation exposure can be evaluated. A previously conducted study found that as the spray quality becomes more fine, the percentage of spray volume within the inhalable range (<100 µm) increases (Flack & Ledson, 2018). This is consistent with another published study demonstrating reduced percentage by volume of droplets below 100 µm using coarse spray quality nozzles (Piggott & Matthews, 1999). Thus, the potential for inhalation exposure for spray applicators is driven by the activities and conditions in which the product is applied.

Currently, inhalation risk assessments do not factor in the human-relevant PSDs that may be inhaled during pesticide handling activities. OVS tubes are standard methods frequently used in pesticide handler exposure studies to measure the amount of particles or aerosol droplets that an operator may be exposed to as an indicator of inhalation exposure. A study conducted by Syngenta showed that OVS tubes sample the inhalable range (<100 µm), with the respirable fraction being a relatively smaller portion of the total inhalation exposure (Flack & Ledson, 2018). While that data set was specific to ground boom nozzles, these findings are consistent with other types of spray applications, including aerial application (Chester & Ward, 1984) and antimicrobial spraying applications with aerosol cans (USEPA, 2012c).

Based on the finding that aerosols captured on OVS tubes fall within the inhalable fraction (<100 µm), a representative distribution for operators applying liquid formulations was mathematically derived using the well-established and internationally recognized sampling conventions established by the International Organization for Standardization (ISO), the American Conference of Governmental Industrial Hygienists (ACGIH) and the Comit Europen de Normalisation (CEN) for the respirable, thoracic and inhalable fractions (TSI, 1997). This "adjusted" inhalable distribution was derived in order to maintain a cut-off of 100 µm while identifying a mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) necessary for inhalation dosimetry models. According to the ISO/ACGIH/CEN sampling conventions, the inhalable particles would include at least 50% of the distribution larger than 100 µm. Hence, the inhalable distribution used in this assessment is health protective by assigning the total airborne concentration to the inhalable fraction. The PSD of aerosols representing all spray application scenarios for the purpose of this refined inhalation approach has an MMAD of 35 µm and GSD of 1.5, and is illustrated graphically in Figure 4. The GSD of 1.5 was assigned to this adjusted inhalable distribution to be consistent with the established definitions for the respirable and thoracic distributions, where both distributions were found to have a GSD of 1.5 (TSI, 1997). For a more detailed discussion of the derivation of size distributions, refer to Flack and Ledson, 2018.

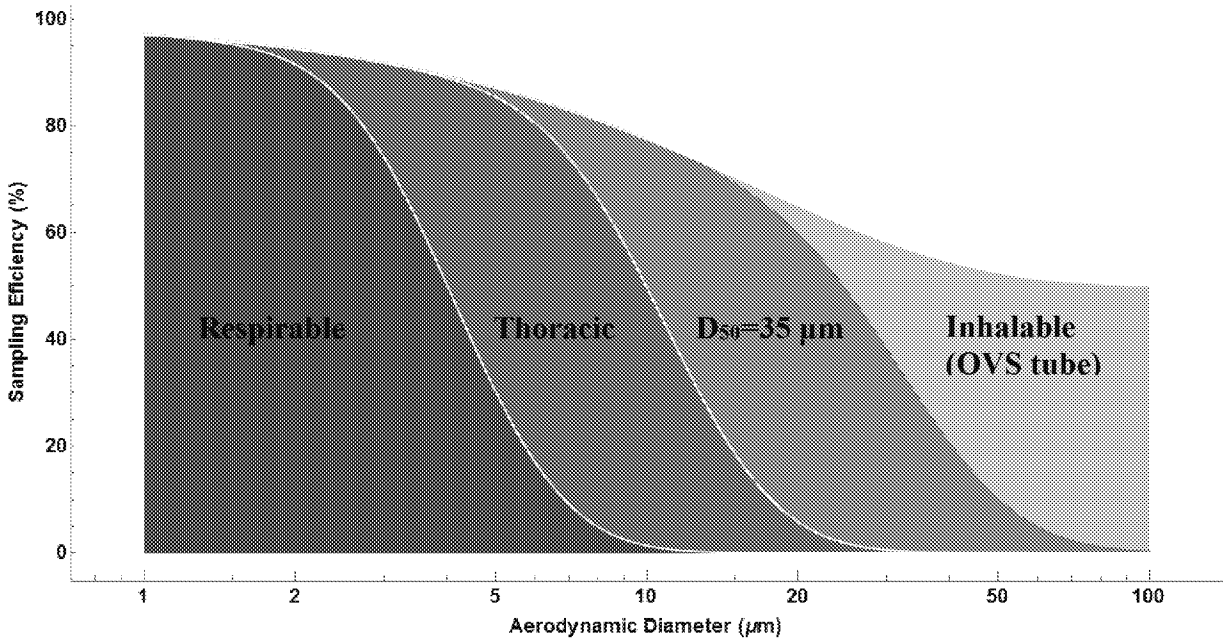


Figure 4 **Derived distribution of health-based particle size selective sampling for respirable (D50 = 4 µm), thoracic (D50 = 10 µm), adjusted inhalable (D50 = 35 µm) and inhalable (<100 µm) fractions based on ISO/CEN/ACGIH sampling conventions.**

The main conclusions for particle characterization of inhaled aerosols are:

1. OVS tubes sample the inhalable fraction (<100 µm).
2. Using well established sampling distribution conventions, a representative PSD was mathematically defined for spray applicators with a cut-off of 100 µm (MMAD = 35 µm, GSD = 1.5).
3. By maintaining a cut-off of 100 µm, the derived representative distribution is both accurate and health protective.

6.0 DOSIMETRY

Dosimetry provides an important link to understanding the relationship between external exposure and biological response. Therefore, accurate dose estimation is critical in understanding dose-response relationships, interspecies extrapolation and risk characterization at given exposure levels. The use of appropriate dosimetry tools improves the accuracy of risk assessments, and reduces the uncertainty of the internal dose estimates at target tissues used to derive the HEC or HED values. They can enable science-based extrapolation of dose across species or studies when animal toxicology or *in vitro* studies are used to identify an effect level. This chapter describes dosimetry models available to estimate deposition in the respiratory tract and summarizes CFD modelling results for CTN in human and rat airways.

6.1 Dosimetry Models

Selection of a dosimetry model to use in inhalation risk assessment depends on the goals of the risk assessment (e.g., screening vs. full risk characterization) and the level of detail and specificity of the data available. What distinguishes dosimetry models, such as RDD, MPPD, and CFD models, is the degree of detail and data underlying different descriptions (Kuempel, Sweeney, Morris, & Jarabek, 2015). One of the earliest models to estimate dose of inhaled particles across species is the USEPA model of the RDD in animal species and human. The calculated RDD ratio (RDDR) is used to adjust the animal deposited dose to an HEC using the inhalation reference concentration (RfC) methodology (USEPA, 1994). However, the RDDR software does not allow for particle size ranges relevant for human exposures, does not provide estimation of particle clearance or retention, nor provide sufficient detail of the upper respiratory tract. The MPPD model is widely-used and freely available, includes both human and rat models of deposition and clearance, and allows for adjustments in particle characteristics (Kuempel, Sweeney, Morris, & Jarabek, 2015). In addition, the MPPD model outputs can be easily integrated into the RfC methodology to derive the HEC (Flack, Bui, & Hinderliter, 2015). Therefore, MPPD should be the first screening tool used when estimating human and rat airway deposition. Like the RDD model, MPPD provides broad regional deposition for the head, tracheobronchial, and pulmonary regions, but also lacks the ability to predict particle deposition in specific sub-regions of the upper respiratory tract, such as the larynx or trachea. Thus, for an upper airway irritant pesticide, such as CTN, with a specific target site, a higher tier dosimetry model is most appropriate.

CFD models are used to predict particle deposition at specific sub-regions of the upper respiratory tract for various particle size ranges of inhaled aerosols. CFD models allow for visualization and estimation of the movement of aerosols to surfaces of tissues within the respiratory tract. The model outputs yield a quantitative estimate of aerosols flowing to and landing on the tissue surfaces, providing exposure values of units of mass per unit area. They have been used in a wide variety of settings from analysing the uptake of reactive gases in different species (USEPA, 2012a) to simulating the deposition of pharmaceutical aerosols in the lungs with *in vivo* or *in vitro* validation (Tian, Hindle, Lee, & Longest, 2015) (Longest, Hindle, Choudhuri, & Byron, 2007). Therefore, model selection for a particular pesticide

active ingredient can be identified through a hierarchical process as the complexity of models and specific data required increases. The benefit of this approach in model selection is the greater precision in the dose estimates, reduction of uncertainty, and more information for decision making.

There are numerous structural differences between the human and rat nasopharyngeal and tracheobronchial regions. Humans have relatively simple noses, while other mammals, including the rat, have complex noses with olfaction as their primary function. Airway dimensions, respiratory parameters and tissue types differ between species, often in ways that do not simply scale with body weight. The differences can be functional, such as a greater ratio of olfactory to respiratory epithelium in rats compared to humans, or physiological, such as the greater nasal surface area (relative to body weight) in rats.

These differences between the human and the rat all modify the air flow, and by extension, the deposition of inhaled particles across their respective respiratory tracts. For example, the sharp bends in the nasopharynx lead to more impaction and interception while the normal upright posture in humans changes the influence of gravitational sedimentation on deposition of aerosols and particles. In addition, different specific regions of the upper respiratory tract have a more simple construction in humans than the rat resulting in differential changes in aerosol droplet or particle direction and velocity. These differences contribute to the reason that the rodent larynx is considered to be more sensitive to effects from inhaled xenobiotics (Mowat, Alexander, & Pilling, 2017; Corley, et al., 2012; Reznik, 1990; Patra, 1986). These biologically significant differences illustrate the advantage of using human-relevant *in vitro* and *in silico* models to refine inhalation risk assessments.

6.2 Computational Fluid Dynamic (CFD) Models for CTN

Three-dimensional CFD models were previously developed for the upper conducting airways of the rat and human (nose through the trachea, (Corley, et al., 2015) (Corley, et al., 2012) (Kabilan, et al., 2016)) and used to calculate the surface concentrations of deposited aerosol formulations of CTN (Corley, Suffield, Kabilan, & Kuprat, 2017).

Deposition of CTN in the respiratory tract is proportional to the % of active ingredient in the product that the worker inhales. Given that applicators will be exposed to diluted products in the tank mix during spraying, the maximum percent of diluted product from Table 1 (4.9% CTN) was used to adjust the CFD dosimetry values. Each simulation for the human model used monodisperse, non-interacting, spherical aerosol particle sizes ranging from 1 to 30 µm at a representative air concentration of 1 mg/L aerosol assuming 4.9% (w/w) CTN formulation.

As the particle size of aerosols increased from 5 µm to 30 µm, deposition in the upper airways also increased from 2.4% to 98.9% of the inhaled particles. For larger aerosol particle sizes, very little penetration past the nose was predicted. In addition, there is evidence that there is less penetration of coarse particulates into the lower respiratory tract than the current size-selective sampling criteria (Brown, Gordon, Price, & Asgharian, 2013). Thus, particles greater than 30 µm were not included in this paper.

Deposition at the 75th percentile is used because it is the highest concentration area in the CFD modelling that is not affected by stochastic variations in the modelling. CFD dosimetry results for CTN at the 75th percentile for the human upper respiratory tract at particle sizes ranging from 1 to 30 µm are summarized in Table 2 (see also Appendix 11.1 for the total aerosol deposition) and the locations for these sub-regions of the respiratory tract are illustrated in Figure 5.

Table 2 Human CFD simulation results for 1 mg/L aerosol, assuming 4.9% (w/w) CTN formulation for aerosol sizes ranging from 1 to 30 µm MMAD.

Aerosol Diameter (µm)	Deposition at 75th Percentile (mg CTN/cm²/breath) adjusted for 4.9% (w/w) CTN					
	Vestibule	Respiratory	Olfactory	Pharynx	Larynx	Trachea
1	5.15E-05	3.66E-05	6.27E-05	2.05E-05	2.59E-05	8.82E-06
3	4.07E-05	2.92E-05	5.44E-05	1.51E-05	2.98E-05	9.26E-06
5	6.86E-05	3.43E-05	1.51E-04	1.78E-05	3.70E-05	7.64E-06
10	1.95E-03	5.39E-05	2.12E-05	6.47E-05	1.68E-04	1.56E-05
15	3.49E-03	3.48E-05	1.17E-05	4.19E-05	1.01E-04	1.68E-05
20	3.31E-03	2.73E-05	7.79E-06	2.22E-05	3.21E-05	6.71E-06
30	1.81E-03	2.27E-05	0.00E+00	6.76E-06	1.23E-05	2.56E-06

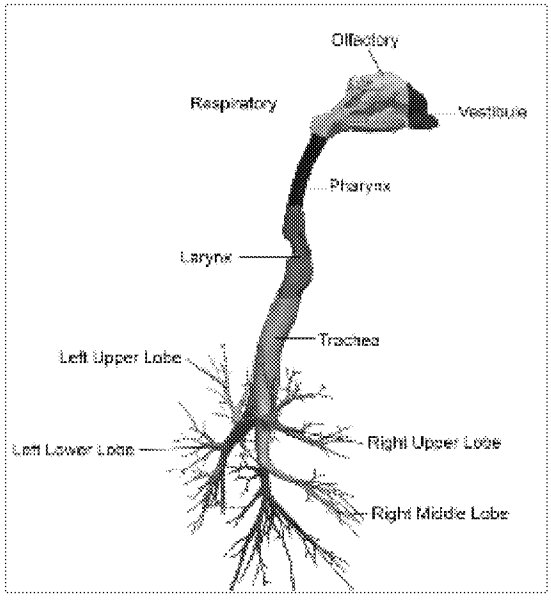


Figure 5 CFD model diagram of human respiratory tract

7.0 OUTCOME

For respiratory irritants, such as CTN, an alternative *in vitro* approach may be taken when *in vivo* data are not adequate or not available to establish a toxicity endpoint for inhalation risk assessment. CTN is classified by the USEPA in toxicity Category II following acute 4 hour exposure via the inhalation route. An adequate *in vivo* subchronic inhalation study was not currently available for CTN. Consequently, an alternative approach using endpoints obtained from MucilAir^{TM}, a three-dimensional *in vitro* model of the human respiratory tract, was used to define CTN inhalation toxicity (Vinall, 2017).

The MucilAir^{TM} system was identified as the optimal *in vitro* model to assess damage to respiratory epithelial cells when compared to alternative *in vitro* models (Table 3, see also (Balogh Sivars, et al., 2017) for a review). This chapter summarizes the MucilAir^{TM} model and describes derivation of the *in vitro* BMDL for CTN (0.00730 mg/cm^2).

Table 3 Available *in vitro* models for assessing damage to respiratory epithelial cells

	Easy to use and to maintain?	Able to model cell-cell interactions in response to toxicants?	Representative of *in vivo* tissue organisation?	Able to simulate the mechanical action of the respiratory system?	Suitable for long term tests?	Assessed for applicability of results to *in vivo* inhalation toxicity?
Immortalised cell monoculture (eg. A549 as reported in Mazzarella et al., 2007)	Yes	No	No	No	No	No
Primary cell monoculture (Ohki et al., 2015)	No	No	No	No	No	No
2D/3D cellular co-culture (eg. Rothen-Rutishauser et al., 2005)	No	Yes	Yes	No	No	No
Lung-on-a-chip (Huh et al., 2010)	Yes	Yes (Limited cell types)	No	Yes	No	No
MucilAir	Yes	Yes	Yes	No	Potentially	Yes (Current Work)

7.1 MucilAir™ *In Vitro* Model

The assay system used in this study, MucilAir™, is a proprietary technology of Epithelix (http://www.epithelix.com/). MucilAir™ is a pseudostratified and ready-to-use 3D model of human airway epithelium, constituted with primary human epithelial cells freshly isolated from nasal, tracheal or bronchial tissues from consent donors upon surgical operations. When seeded onto a semi-porous membrane (Costar Transwell, pore size 0.4 μm) at the air-liquid interface, the de-differentiated cells undergo a progressive differentiation and polarization (both morphologically and functionally) to a fully ciliated epithelia (Figure 6). The mature MucilAir™ is composed of basal cells, ciliated cells and mucus cells. The proportion of these various cell types is preserved compared to *in vivo* observation (Huang, Wiszniewski, & Constant, 2011). The cells recapitulate the pseudostratified ciliated respiratory epithelium with goblet cells that are normally found lining parts of the nasal cavity and the larynx, trachea, bronchi, and bronchioles *in vivo* in mammals. Histologically, the relative distribution of squamous, respiratory, and olfactory epithelium from the nostrils to the nasopharynx is similar between humans and other mammals. Although the dimensions of the passageways through which the air flows in the respiratory tract are dramatically different across species such as the human and the rat, the thickness of the mucus and epithelial layer is the same (0.0065 cm) (Reznik, 1990; Corley, 2012). The initial effect related to exposure at the site of adverse response regardless of model system would likely be the same. Thus, the Mucilair™ assay system would recapitulate what is occurring at the epithelial/mucus/air interface.

Once differentiated, MucilAir™ can be kept in a homeostatic state for several months. The assay system can be used to measure a variety of membrane and cell damage endpoints as markers of cellular response to chemicals including irritants.

Nasal, tracheal and bronchial epithelia are all pseudo-stratified and made of the same three types of cells (basal, ciliated and goblet cells). Nasal epithelium is quite often used as a surrogate of bronchial epithelium due to easy availability from nasal brushings for example. Some comparative studies between MucilAir Nasal and Bronchial suggest that their respective responses to xenobiotics is equivalent (Iskandar, 2013). This is also the case for functional assays such as trans epithelial permeability of xenobiotics (Hoffmann, et al., 2018).

MucilAir™ is functionally differentiated, secretes mucus and has electrically tight junctions (TEER>200 $\Omega.cm^2$). The activity of the main epithelial ionic channels, such as CFTR, EnaC, Na/K ATPase, is preserved, and the epithelia is shown to respond in a regulated and vectorial manner to the pro-inflammatory stimulus, TNF-α (Huang, Wiszniewski, & Constant, 2011). A large panel of cytokines, chemokines and metalloproteinases has been detected in MucilAir™ (e.g. IL-8, IL-6, GM-CSF, MMP-9, GRO-α). Most importantly, MucilAir™ replicates the main function of the airway epithelial cells, mucociliary clearance driven by synchronized cilia-beating.

Cells used for the reconstitution of MucilAir™ are tested negative for Hepatitis B & C and HIV-1 & 2. All the ALI cultures produced are tested twice per week for sterility including bacterial, fungus and mycoplasma. Each batch of MucilAir™ produced is subjected to comprehensive quality control. Optimal differentiation of the epithelium is monitored by the presence of cilia and tight junctions ensuring good cilia beating frequency and TEER. Furthermore, pseudo-stratified architecture of the epithelium is verified by histology (H/E – Alcian Blue staining). A certificate of analysis is provided for each batch of MucilAir™.

MucilAir™ is applicable for acute, long-term and chronic *in vitro* studies including, but not limited to, toxicity testing, viral and bacterial infections, and respiratory diseases like asthma and COPD (Constant, Wisniewski, & Huang, 2014), (Tapparel, et al., 2013), (Balogh Sivars, et al., 2017), (Hoffmann, et al., 2018).

Publications using the MucilAir™ model (more than one hundred as of this writing) are available at: http://www.epithelix.com/support/publications. This demonstrates the quality, robustness, usefulness, and usability of the MucilAir™ model for various applications including toxicity testing and dose-response evaluation.

The endpoints evaluated in this work for CTN dose-response are briefly described below:

1. **Trans-epithelial electrical resistance (TEER)**: measures the integrity of tight junctions between cells in the membrane.
2. **Lactate dehydrogenase (LDH)**: an enzyme present in most cells and released when cells suffer sufficient membrane damage to indicate cytotoxicity leading to cell death.
3. **Resazurin metabolism**: reduced to a fluorescent product in viable cells and used as a measure of metabolic competence.

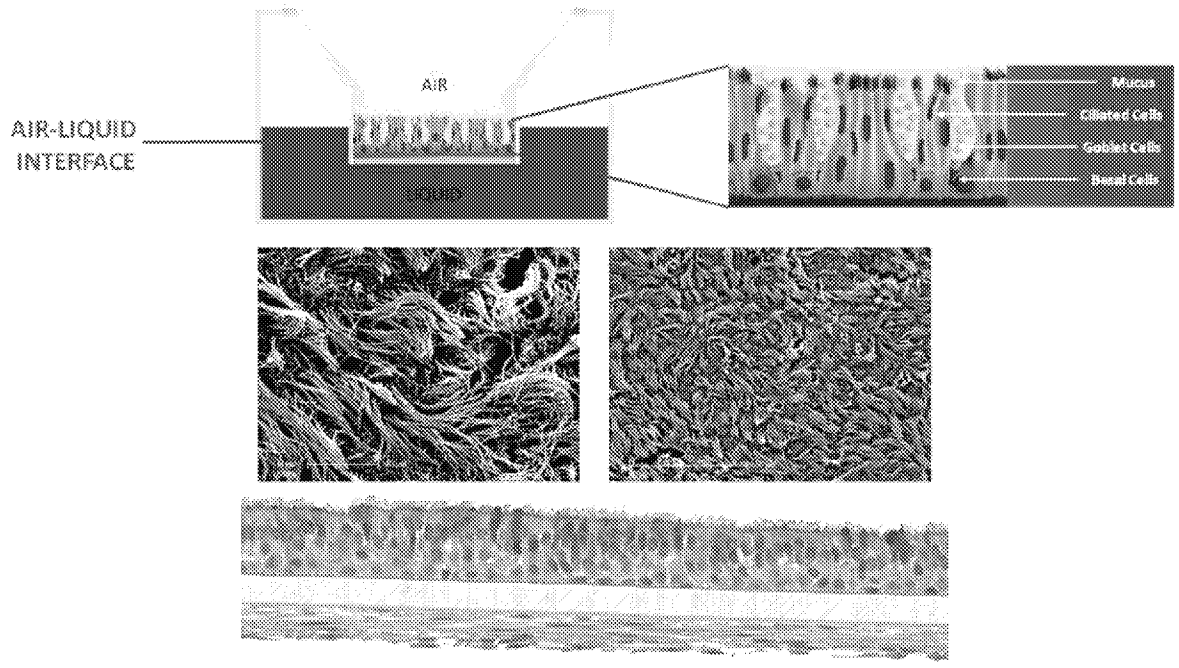


Figure 6 **Schematics of the MucilAir™ *in vitro* system.**

The system consists of human epithelial cells on a transwell plate with air and liquid interfaces (top panel). The differentiated cells form a pseudostratified ciliated respiratory epithelium (top right). Electron microscopy shows the cell configuration (at 10 and 50 µm, center), animations of cilia movement are available on the Epithelix website at http://www.epithelix.com/support/videos. After 45 days of culture at air-liquid interface, the epithelia were fully differentiated, both morphologically and functionally (longitudinal slide, bottom). Figures taken from www.epithelix.com.

The *in vitro* assay is able to recapitulate the adverse outcome pathway that occurs *in vivo* and would be the same regardless of species because of the nature of the initiating event (Figure 7). The adverse outcome pathway is initiated on exposure of the respiratory epithelial cell to the irritant CTN, which damages the epithelial cell, leading to changes in TEER and metabolism and leakage of LDH *in vitro*, and is seen histologically as cell death or necrosis *in vivo*. After persistent exposure or repeated exposure, the repeated cell death of the lining epithelium results in a metaplastic response that serves to protect the respiratory tract from the irritant effects. After days of repeated exposure, the respiratory epithelium transforms into stratified squamous epithelium, which becomes resistant to the irritant effect of CTN and thus protects the respiratory tract from further damage. It is well accepted that the rodent larynx is particularly sensitive to aerosol irritant damage. The most common alteration in the larynx is squamous metaplasia (Mowat et al., 2017). This response is dependent on a sufficient concentration of CTN at the cell surface to result in cell death. Thus, the *in vitro* assay mirrors what is happening *in vivo* at the initial interaction between the CTN and the respiratory cell. It is, therefore, reasonable to extrapolate toxicodynamic effects on the respiratory epithelium directly from the *in vitro* assay to the *in vivo* situation in the rodent or human.

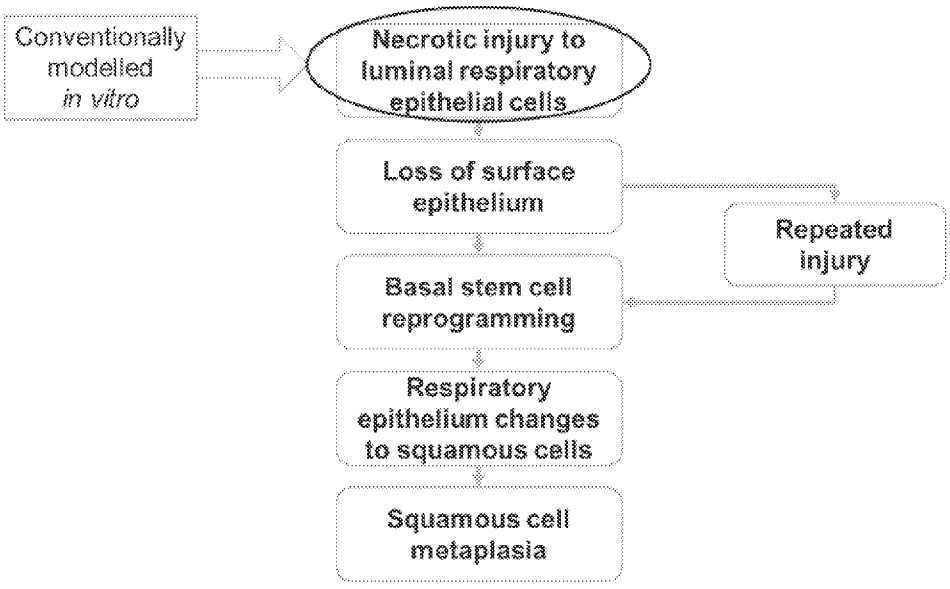


Figure 7 **Adverse outcome pathway for irritant induced laryngeal squamous metaplasia. (Adapted from: Renne et al. 2009)**

The CTN dose-response data were collected for each of the three endpoints (LDH, TEER and resazurin) among 5 separate donors, 10 dose levels ranging from 2 to 200 mg/L, and 6 replicates per dose. CTN was applied as the Bravo 720 formulation (54.7% chlorothalonil w/w) to MucilAir^{TM} tissues and exposed for a period of 24 hours (Vinall, 2017).

7.2 Benchmark Dose (BMD) and BMDL

MucilAir^{TM} dose-response data for CTN were analysed using the Benchmark Dose Software (BMDS) developed by USEPA. A detailed description and results of this analysis are provided in the study report (Li & Brambilla, 2017). TEER, LDH and resazurin metabolism were measured following treatment. For each endpoint (TEER, LDH and resazurin), the benchmark dose (BMD) and the lower-bound of the 95% confidence limit (BMDL) were estimated using a constant variance Hill model. In order to comply with EPA guidance on the calculation of BMD values, BMD_{sd} values were calculated (Li & Brambilla, 2017) and used in this document (descriptive statistics of the dose-response data and calculated values are presented in Appendix 11.3). The three endpoints responded similarly to CTN challenge and showed small, but biologically insignificant, variations among the five donors, suggesting low inter-donor variability in sensitivity. The BMDL values were in the 40 – 125 mg/L range (Table 4) and when translated to mass per unit surface area, concentrations were 0.0038 – 0.0112 mg/cm^2 (Table 5). An overall mean BMDL value of 0.00730 mg/cm^2 was established as the inhalation toxicity endpoint for CTN.

Table 4 Chlorothalonil BMDL values in mg/L calculated from MucilAir data.

Donor	BMDL (mg/L)			
	TEER	LDH	Resazurin	Geometric Mean
1	51.21	67.60	66.72	**61.36**
2	53.09	92.85	80.47	**73.48**
3	88.24	90.15	91.85	**90.07**
4	110.20	91.00	42.45	**75.23**
5	124.40	101.60	113.2	**112.68**
Geometric Mean	**80.06**	**87.85**	**74.98**	**80.79**

As illustrated in Figure 6, MucilAir™ tissue inserts are cultured within the wells of a 24 well plate. The internal diameter of the MucilAir™ tissue culture inserts is 6.5mm (33.18 mm^2), 30 µL of test material is uniformly applied to the surface of this insert. As such, BMDL values in mg chlorothalonil/L were converted to tissue concentration BMDL values in mg chlorothalonil/cm^2 using the equation below:

$$\text{BMDL}\ (\text{mg/cm}^2) = \text{BMDL}\ (\text{mg/L}) \times \frac{30\ \mu\text{L} \times 1\text{x}10^{-6}\ \text{L}/\mu\text{L}}{33.18\ \text{mm}^2 \times 0.01\ \text{cm}^2/\text{mm}^2} \qquad \text{Equation 1}$$

Table 5 Chlorothalonil BMDL values in mg/cm^2 calculated from MucilAir data.

Donor	BMDL (mg/cm^2)			
	TEER	LDH	Resazurin	Geometric Mean
1	0.00463	0.00611	0.00603	**0.00555**
2	0.00480	0.00840	0.00728	**0.00664**
3	0.00798	0.00815	0.00830	**0.00814**
4	0.00996	0.00823	0.00384	**0.00680**
5	0.01125	0.00919	0.01024	**0.0102**
Geometric Mean	**0.00724**	**0.00794**	**0.00678**	**0.00730**

8.0 OPERATOR RISK ASSESSMENTS

This chapter describes calculation of the HEC for CTN using site-specific deposition estimates for the upper human respiratory tract and *in vitro* inhalation endpoint. An evaluation of DDEFs, as well as use of the HEC value in inhalation risk assessment for occupational spray applicator scenarios is discussed.

8.1 Calculating the Human Equivalent Concentration (HEC)

This section describes the use of PSDs for human exposures, human dosimetry from the CFD model, and the MucilAir^{TM} BMDL to derive an HEC for CTN inhalation risk assessments. The CFD model assumed an external exposure aerosol concentration of 1 mg/L and estimated deposition for discrete particle sizes ranging from 1 to 30 µm (Section 6.2). A BMDL of 0.00730 mg CTN/cm^2 was identified from the MucilAir^{TM} model (Section 7.2). Summary of the human-relevant parameters needed for calculating the HEC for spray applicators (i.e., PSDs, breathing rates and exposure durations) are summarized in Table 6. The breathing rate of 12.7 breaths/min and tidal volume (L) were calculated from the minute ventilation (8.3 L/min) keeping the ratio of breathing frequency and tidal volume constant for light and moderate level activities (de Winter-Sorkina & Cassee, 2002). The minute ventilation of 8.3 L/min is the value used by AHETF to calculate inhalation exposure for spray applicators who are sedentary (e.g., driving a tractor). A supporting document summarizing the breathing rate (breaths/minute) calculation is provided in Appendix 11.2.

Table 6 Summary of human-relevant parameters used in calculating the Human Equivalent Concentration (HEC)

Exposure Scenario	Particle Size Distribution (MMAD, GSD)	Breathing Rate (breaths/minute)[1]	Exposure Duration (h)[2]	Total Breaths
Spray Applicator	35 µm, 1.5	12.7	8	6,096

[1] Calculated from AHETF breathing rate (8.3 L/min) for spray applicators according to de Winter-Sorkina and Cassee, 2002. Calculations are provided in Appendix 11.2.
[2] EPA default exposure duration for occupational operators.

Calculating the HEC can be summarized in the following steps:

1. Calculate the cumulative site-specific deposition of polydisperse particles for the MMAD of 35 µm and GSD of 1.5.
2. Calculate the total deposition of active ingredient over the relevant exposure duration.
3. Calculate the HEC using the BMDL and total deposition of active ingredient.

8.1.1. Cumulative Site-Specific Deposition of Polydisperse Particles.

As shown in Chapter 5.0, operators are exposed to distributions of particle sizes (polydisperse) and not to discrete particle sizes (monodisperse). However, the CFD model estimates deposition for discrete particle sizes. Therefore, to calculate deposition of polydisperse particles, the deposition values from the CFD model at the 75th percentile for discrete particle sizes (Table 2) and the percent contribution of each discrete particle size to the relevant MMAD (35 μm) and GSD of 1.5 were used to calculate the cumulative deposition across different sized particles in site-specific regions of the respiratory tract.

The calculation of percent contribution of each discrete particle size to the continuous distributions of particle sizes can be mathematically described as follows. The PSDs defined in Section 5.0 for applicators are probability density functions (pdf) and associated with continuous rather than discrete random variables. The values of these continuous PSDs are not probabilities; therefore, the probability mass function (pmf) needs to be determined to yield the probability for each discrete random particle size.

Let f(x) denote the probability mass function defined on a sample space S and if X is a discrete random variable. The pmf satisfies the following properties:

$$\boldsymbol{P(X = x) = \begin{cases} f(x) > 0, & for\ x \in S \\ 0, & x \notin S \end{cases}} \qquad \text{Equation 2}$$

and total probability for all outcomes x is given

$$\boldsymbol{\sum_{x \in S} f(x) = 1} \qquad \text{Equation 3}$$

Using the definition of $I_A(x)$ (inhalable fraction) and the above properties of pmf, the constant c will be determined so that the function f(x) satisfies the conditions of being a probability mass function.

$$\boldsymbol{\sum_{i=1}^{7} c\ I_A(x_i) = 1,\ x_i \in \{1, 3, 5, 10, 15, 20, 30\}} \qquad \text{Equation 4}$$

For the PSD used here ($I_A(x)$ with MMAD = 35 μm (GSD 1.5), c=17.327. The percent contribution for each relevant particle size is calculated using the corresponding normalization constant. These results are summarized in Table 7. The discontinuous probability mass functions are displayed in Figure 8 which shows the probability associated with particle diameters for the relevant PSDs (MMAD = 35 μm, GSD = 1.5).

Table 7 Percent contribution of discrete particles to the relevant particle size distributions (MMAD = 35, GSD = 1.5)

Aerosol Diameter (µm)	Percent Contribution MMAD = 35 µm, GSD = 1.5
1	<0.0001% (3.43×10^{-14} %)
3	<0.0001% (6.06×10^{-6} %)
5	0.0034%
10	1.44%
15	12.80%
20	32.89%
30	52.87%

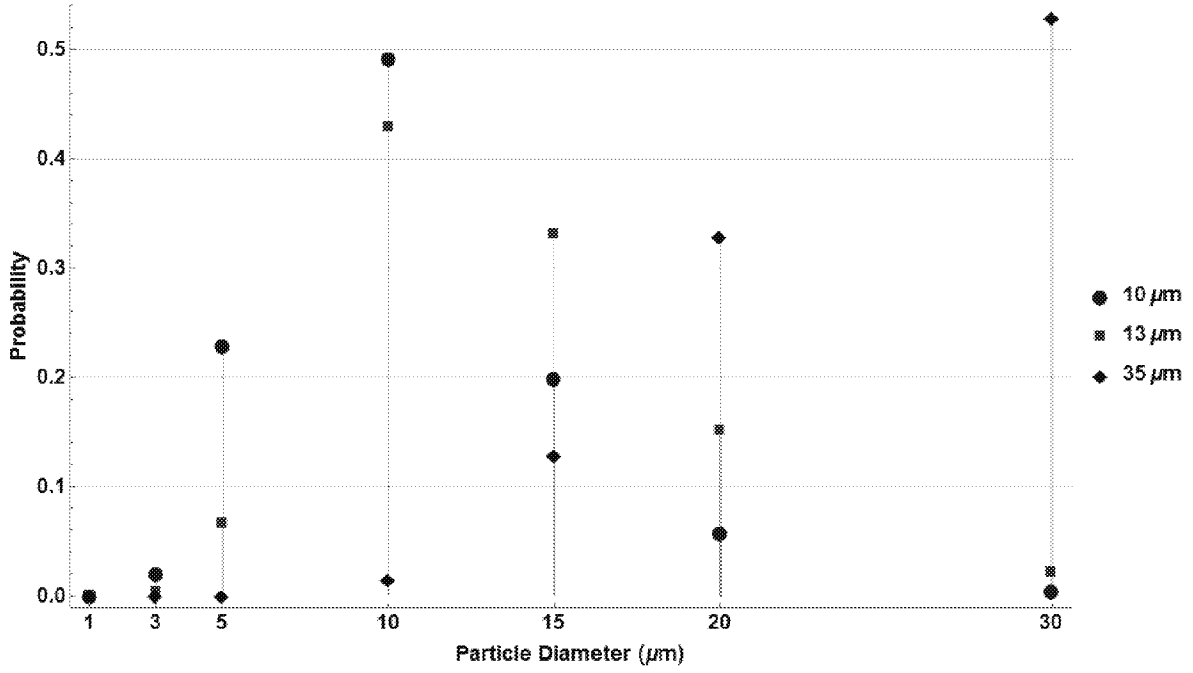


Figure 8 Discrete probability mass functions for the relevant particle size distributions

The cumulative deposition across different sized aerosols in a given site-specific region of the respiratory tract can be calculated utilizing the percentage of inhaled aerosols deposited across different particle sizes (Table 7), or relevant probability mass functions as follows:

$$\sum_{i=1}^{7} \boldsymbol{f}(x_i) \times \boldsymbol{Deposited}(x_i), \quad x_i \in \{1, 3, 5, 10, 15, 20, 30\} \qquad \text{Equation 5}$$

where $\boldsymbol{Deposited}(x_i)$ is the deposition value for the airway region at the 75th percentile from Table 2. These summations were carried out for the respiratory, olfactory, pharynx, larynx, and trachea regions. An example of this calculation is summarized in Table 8 where deposition is calculated for each discrete particle size in the larynx corresponding to MMAD of 35 µm (GSD = 1.5) and summed to yield the cumulative particle deposition (3.56E-04 mg/cm^2/breath).

Table 8 Calculation of the cumulative particle deposition for 1 mg/L aerosol in the Larynx for MMAD = 35 µm, GSD = 1.5 assuming 4.9% (w/w) CTN formulation

Aerosol Diameter (µm)	Deposition in Larynx[1] (mg CTN/cm^2/breath) (monodisperse)	Percent Contribution[2] (MMAD = 35 µm, GSD = 1.5)	Deposition in Larynx[3] (mg CTN/cm^2/breath) (MMAD = 35 µm, GSD = 1.5)
1	2.59E-05	<0.0001% (3.43×10^{-10}%)	8.90E-17
3	2.98E-05	<0.0001% (6.06×10^{-6}%)	1.81E-12
5	3.70E-05	0.0034%	1.26E-09
10	1.68E-04	1.44%	2.42E-06
15	1.01E-04	12.80%	1.30E-05
20	3.21E-05	32.89%	1.06E-05
30	1.23E-05	52.87%	6.53E-06
Cumulative (total)	NA	100%	**3.25E-05**

[1] Deposition amount at 75th percentile in human larynx from CFD simulation. (see Table 2)
[2] Percent contribution of discrete particle to PSD (MMAD=35 µm, GSD = 1.5) (see Table 7)
[3] Deposition (mg CTN/cm^2/breath) = Deposition (monodisperse) × percent contribution/100

While the CFD model captures deposition in the nasal vestibule, this region was not considered relevant to the inhalation risk assessment. The nasal vestibule is the most anterior part of the nasal cavity and is lined by the same epithelium as human skin (stratified squamous epithelium), as well as small hairs which act to filter dust and other material to prevent inhalation of those materials. Therefore, this region functions similarly to skin epidermis to protect underlying tissues from potentially harmful environmental agents (Harkema, Carey, & Wagner, 2012). These cell types are very different from those within the other regions of the upper respiratory tract (i.e., respiratory epithelium) including the cell types used in the MucilAir^{TM} assay for deriving the BMDL. Thus, the vestibule was not included among the regions used to calculate the HEC.

Applying the same calculation for the relevant sub-regions of the upper respiratory tract yields cumulative particle deposition values for spray applicator scenarios (Table 9). The region of the respiratory tract receiving the highest deposition values is the larynx.

Table 9 Cumulative particle deposition for 1 mg/L aerosol in site-specific regions of the respiratory tract for each exposure scenario assuming 4.9% (w/w) CTN formulation

Exposure Scenario	Cumulative Deposition Amount (mg CTN/cm^2/breath)				
	Respiratory	Olfactory	Pharynx	Larynx	Trachea
Spray Applicator	2.62E-05	4.37E-06	1.72E-05	3.25E-05	5.93E-06

8.1.2 Total Deposition of Active Ingredient over the Exposure Duration

Using the cumulative deposition values (
Table 9), breathing rate and exposure duration (Table 6), the total daily CTN deposition for each exposure scenario was calculated according to the following equation:

$$DepT = DepB \times BR \times Exp \times CF \qquad \text{Equation 6}$$

Where,
DepT = total daily deposition (total mg CTN/cm^2)
DepB = cumulative deposition per breath for 1 mg/L aerosol (mg CTN/cm^2/breath) (see Table 9)
BR = breathing rate (12.7 breaths/min)
Exp = exposure duration (8 hours)
CF = conversion factor (60 min/hr)

The total deposition of CTN over the exposure duration for each site-specific region of the respiratory tract is summarized in Table 10.

Table 10 Total deposition of CTN in site-specific regions of the respiratory tract for each exposure scenario assuming 4.9% (w/w) CTN formulation

Exposure Scenario	Total Deposition Amount (mg CTN/cm^2)				
	Respiratory	Olfactory	Pharynx	Larynx	Trachea
Spray Applicator	0.16	0.027	0.10	0.20	0.036

8.1.3 Human Equivalent Concentration (HEC)

The HEC can be extrapolated from the BMDL, established by the MucilAir^{TM} model, using the daily total deposition for a 1 mg/L aerosol concentration according to the following equation:

$$HEC = \frac{BMDL}{DepT} \times AC \qquad \text{Equation 7}$$

Where,
HEC = Human Equivalent Concentration (mg/L)
BMDL = Benchmark Dose Level (0.00730 mg CTN/cm^2) (see Table 5)
DepT = total daily deposition (mg CTN/cm^2) (see Table 10)
AC = aerosol concentration (1 mg/L)

A summary of the HEC values corresponding to the spray application of liquids exposure scenario is summarized in Table 11.

Table 11 Human Equivalent Concentration (HEC) values assuming 4.9% (w/w) CTN formulation

Exposure Scenario	Human Equivalent Concentration (mg/L)				
	Respiratory	Olfactory	Pharynx	Larynx	Trachea
Spray Applicator	0.046	0.27	0.070	0.037	0.20

8.2 Data-Derived Extrapolation Factors

USEPA has provided guidance on the calculation of DDEFs (USEPA, 2014) for use in place of traditional uncertainty factors. The traditional 10X interspecies and 10X intraspecies extrapolation factors are replaced with:

$$CF = EF_{AK} \times EF_{AD} \times EF_{HK} \times EF_{HD} \qquad \text{Equation 8}$$

Where:
CF = Composite Factor
EF_{AK} = interspecies toxicokinetic (TK) extrapolation factor
EF_{AD} = interspecies toxicodynamic (TD) extrapolation factor
EF_{HK} = intraspecies toxicokinetic (TK) extrapolation factor
EF_{HD} = intraspecies toxicodynamic (TD) extrapolation factor

The work presented in this Source to Outcome approach has informed several of these factors. Both of the interspecies factors (EF_{AK} and EF_{AD}) are reduced to 1. EF_{AK} is set to 1 because the CFD dosimetry model directly calculates the human dose at the target site. EF_{AD} is set to 1 because the measured endpoint (BMDL) was conducted in a human derived system (respiratory epithelial tissue) and thus the TD response is directly measured in humans. While the MucilAir evaluation was conducted on 5 individuals and the CFD is applicable across individuals, sufficient information is likely not currently available to change EF_{HK} x EF_{HD} from the default value of 10.

Two other additional uncertainty factors have previously been included in the CTN risk assessment: a database 3x UF for lack of a NOAEL and a 10x for exposure duration. Because this approach calculated a BMDL, the database uncertainty factor can be excluded. Because a typical daily operator exposure occurs for 8 hours in occupational settings and the daily exposure duration in the *in vivo* rat study is 6 hours, deriving a point of departure based on a 24 hour exposure duration from the *in vitro* assay would be health protective and the exposure duration UF is unnecessary. Therefore, for operator risk assessments, the net LOC would be 10 (Table 12).

Table 12 Uncertainty Factors for Risk Assessment

Uncertainty Factor	Prior Value[1]	Recommended Value
Inter-species variability	3	1
Intra-species variability	10	10
No NOAEL	3	1
Exposure duration	10	1

[1] Uncertainty factors identified in USEPA draft human health risk assessment for CTN (USEPA, 2012b).

8.3 Occupational Exposure Risk Assessment

To illustrate the Source to Outcome Approach, risk estimates (MOEs) were calculated for different spray applicator scenarios. These spray applicator scenarios would also represent the highest exposed populations among these application types, and thus would be protective of other applicator scenarios. Therefore, using the most health protective HEC values, which were specified for the larynx in Table 11 (0.037 mg/L), resulted in short- and intermediate-term MOEs ranging from 174 to 17,364 for representative spray applicator scenarios without additional respiratory protective equipment (RPE) (Table 13).

Table 13 CTN inhalation exposures (mg/L) and MOE results for representative spray application scenarios

Exposure Scenario	Crop/Use site	Max Application Rate on Label[1]	Area treated	Inhalation Unit Exposure[2]	Inhalation Exposure[3]	Short- and Intermediate-term MOEs[4]
		(lb ai/A)	(Acres)	(mg ai/lb handled)	(mg/L)	No RPE
Spray Applicator						
Aerial application	Soybean	1.8	1,200	4.9E-06	2.68E-06	14,000
Aerial application	Cranberries	5.0	350	4.9E-06	2.13E-06	17,000
Airblast application	Pistachio	4.5	40	4.7E-03	2.13E-04	170
Airblast application	Stone Fruit	3.1	40	4.7E-03	1.46E-04	250
Groundboom application	Golf Course	11.3	40	3.4E-04	3.86E-05	960
Groundboom application	Sod Farm	11.3	80	3.4E-04	7.71E-05	480

[1] Maximum use rates from Syngenta labels: Bravo Weather Stik (50534-188-100), Daconil Weather Stik (50534-209-100).

[2] Inhalation Unit Exposure (mg/lb ai) values for no RPE (baseline) or engineering controls (enclosed cockpit) for aerial application (EPA Occupational Pesticide Handler Unit Exposure Surrogate Reference Table, November 2016)

[3] Inhalation exposure (mg/L) = [Unit Exposure (mg/lb ai) × Max App Rate (lb ai/A) × Acres/day] ÷ (8.3 L/min × 60 min/hr × 8 hr/day).

[4] ST/IT MOEs = HEC (larynx) [0.037 mg/L] ÷ Inhalation exposure (mg/L)

The range of HEC values (37 – 270 µg/L) from Table 11 can be plotted against these range of exposure values across the spray applicator scenarios (Table 13) with an LOC of 10 as a point of reference (Figure 9). These results demonstrate that risks to spray operators do not exceed the Level of Concern (LOC of 10). The resulting MOEs for these applicator scenarios show that exposure levels are within acceptable limits, resulting in reasonable certainty of no harm to the spray applicators.

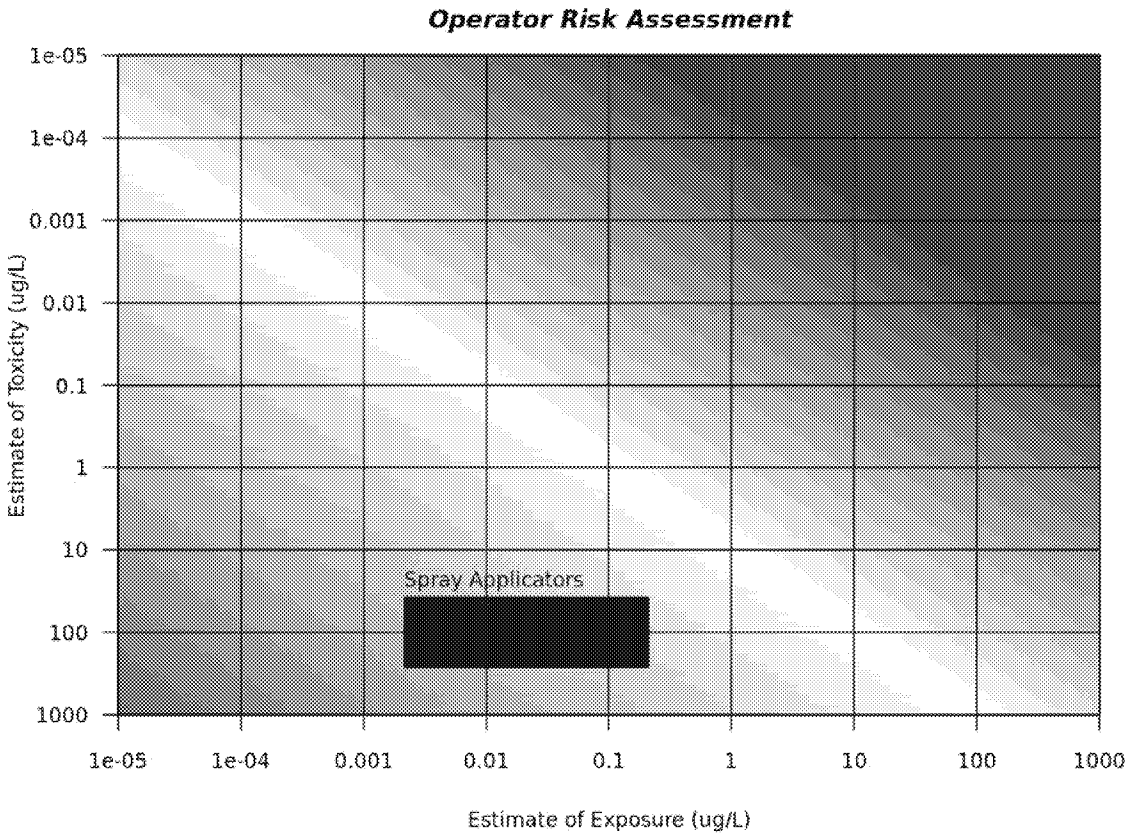


Figure 9 **Risk21 plot (Toxicity versus Exposure) for Spray Applicator Risk Assessment for CTN.**

9.0 CONCLUSION

The Source to Outcome Approach yielded more precise and accurate respiratory dose estimates for operators applying liquid sprays, resulting in CTN risk assessments that are precise, accurate and health protective. This same approach could also be used for other operator scenarios for CTN, such as mixing/loading, by integrating appropriate PSDs and assumptions for % CTN relevant for these scenarios. This approach resulted in several important findings pertaining to CTN inhalation risk assessment:

1. OVS tubes sample the inhalable fraction (<100 μm), with PSD of aerosols identified relevant for human exposures.
2. The *in silico* (CFD) model yielded dosimetry estimates for the human upper respiratory tract in the critical sub-regions of interest (e.g., larynx).
3. The *in vitro* (MucilAir^{TM}) study established a human BMDL (0.00730 mg/cm^2).
4. The elements of the source to outcome approach allow the calculation of DDEFs in place of standard uncertainty factors.
5. The HEC extrapolated from the *in vitro* BMDL resulted in risk assessments for spray applicators that were not of concern (i.e., MOEs> LOC of 10).

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11.0 APPENDICES

11.1 Supporting Aerosol Deposition Tables

Table 11.1a Human CFD simulation results for 1 mg/L aerosol, total deposition for aerosol sizes ranging from 1 to 30 µm MMAD.

Aerosol Diameter (µm)	Deposition at 75th Percentile (mg/cm²/breath)					
	Vestibule	Respiratory	Olfactory	Pharynx	Larynx	Trachea
1	1.05E-03	7.46E-04	1.28E-03	4.19E-04	5.29E-04	1.80E-04
3	8.30E-04	5.95E-04	1.11E-03	3.09E-04	6.08E-04	1.89E-04
5	1.40E-03	7.01E-04	3.09E-03	3.63E-04	7.55E-04	1.56E-04
10	3.98E-02	1.10E-03	4.33E-04	1.32E-03	3.43E-03	3.19E-04
15	7.12E-02	7.10E-04	2.38E-04	8.56E-04	2.07E-03	3.42E-04
20	6.76E-02	5.57E-04	1.59E-04	4.53E-04	6.55E-04	1.37E-04
30	3.69E-02	4.63E-04	0.00E+00	1.38E-04	2.52E-04	5.22E-05

Note: These values are total aerosol deposition in the respiratory tract and are taken from the 75th percentile deposition columns (across all simulated aerosol diameters) from Table 5 of the CFD report (Corley, Suffield, Kabilan, & Kuprat, 2017)

Table 11.1b Human CFD simulation results for 1 mg/L aerosol, assuming 54.7% (w/w) CTN formulation for aerosol sizes ranging from 1 to 30 µm MMAD.

Aerosol Diameter (µm)	Deposition at 75th Percentile (mg CTN/cm²/breath)					
	Vestibule	Respiratory	Olfactory	Pharynx	Larynx	Trachea
1	5.74E-04	4.08E-04	7.00E-04	2.29E-04	2.89E-04	9.85E-05
3	4.54E-04	3.25E-04	6.07E-04	1.69E-04	3.33E-04	1.03E-04
5	7.66E-04	3.83E-04	1.69E-03	1.99E-04	4.13E-04	8.53E-05
10	2.18E-02	6.02E-04	2.37E-04	7.22E-04	1.88E-03	1.74E-04
15	3.89E-02	3.88E-04	1.30E-04	4.68E-04	1.13E-03	1.87E-04
20	3.70E-02	3.05E-04	8.70E-05	2.48E-04	3.58E-04	7.49E-05
30	2.02E-02	2.53E-04	0.00E+00	7.55E-05	1.38E-04	2.86E-05

Note: These values would correspond to the undiluted Bravo 720 formulation used in the MucilAir *in vitro* experiments.

11.2 Supporting Calculations for Breathing Rate (breaths/min)

Minute Ventilation (L/min)	Breathing Rate (1/min)	Tidal Volume (L)	Ratio (BR/TV)	
5	10	0.50	20.0	
8	12	0.63	19.2	
13	16	0.81	19.7	
19	19	1.00	19.0	
25	22	1.14	19.4	
30	24	1.25	19.2	
35	26	1.35	19.3	
40	28	1.43	19.6	
59	34	1.74	19.6	
8.3	**12.7**	**0.65**	**19.4**	<--average

Source: de Winter-Sorkina and Cassee, 2002

Calculations:

MV / BR = TV
TV * BR = 8.3
BR/TV = 19.4
BR = 19.4 * TV
TV * (19.4 * TV) = 8.3
TV^2 = 8.3/19.4

TV= 0.65 L

MV = minute ventilation (L/min)
BR = breathing rate (1/min)
TV = tidal volume (L)

BR = MV/TV
BR = 8.3/0.65

BR = 12.7 breaths/min

11.3 Descriptive Statistics of Mucilair Dose-Response Data and Calculated BMD Values

Table A1.1. Descriptive statistics of the three endpoints for the five donors with six replicates at each dose level in the MucilAir data

Dose Level		LDH (relative units)					TEER (Ω)					Resazurin (% NC)				
CTN (mg/L)	log10 CTN	N	Mean	SD	Min	Max	N	Mean	SD	Min	Max	N	Mean	SD	Min	Max
NC*	0.0	30	1.2	1.4	0.0	4.1	30	1219	333.5	580.0	1651	30	100.0	21.2	64.8	141.8
2.00	0.3	30	2.5	3.5	0.0	11.8	30	1211	282.8	704.0	2021	30	190.9	30.9	118.4	246.5
5.01	0.7	30	0.9	1.3	0.0	3.9	30	1193	304.0	556.0	1755	30	152.2	38.2	86.1	230.4
7.94	0.9	30	0.8	1.2	0.0	4.3	30	1181	360.2	342.0	1842	30	134.0	32.2	82.2	207.4
12.6	1.1	30	0.9	1.2	0.0	4.9	30	1290	360.6	524.0	1803	30	156.1	31.1	97.1	228.3
19.9	1.3	30	1.1	1.3	0.0	6.1	30	1216	335.4	538.0	1683	30	177.0	32.5	127.4	257.1
31.6	1.5	30	0.7	0.9	0.0	3.2	30	1166	334.4	571.0	1734	30	132.5	35.3	66.4	201.4
50.1	1.7	30	0.9	1.3	0.0	4.8	30	1204	353.9	540.0	1590	30	134.2	41.3	69.2	220.2
79.4	1.9	30	1.0	1.4	0.0	4.5	30	1161	348.6	544.0	1823	30	136.5	20.6	96.6	189.9
126	2.1	30	16.2	31.6	0.0	96.5	30	845.2	474.7	123.0	1564	30	105.9	25.0	56.2	156.2
199	2.3	30	168.8	47.5	86.3	286.6	30	123.4	29.0	65.0	194	30	20.8	23.2	-0.0	68.4

* Negative control

Table A1.2. Means, standard deviations (STD) and coefficients of variation (CV) of BMD and 95% BMDL

Study donor no.	LDH				TEER				Resazurin			
	BMD log10	BMD	BMDL log10	BMDL	BMD log10	BMD	BMDL log10	BMDL	BMD log10	BMD	BMDL log10	BMDL
1	1.850	70.74	1.830	67.60	1.750	56.26	1.709	51.21	1.916	82.43	1.824	66.72
2	1.985	96.62	1.968	92.85	1.910	81.24	1.725	53.09	2.081	120.5	1.906	80.47
3	1.973	93.96	1.955	90.15	1.988	97.17	1.946	88.24	2.082	120.9	1.963	91.85
4	1.979	95.25	1.959	91.00	2.064	115.9	2.042	110.2	1.900	79.41	1.628	42.45
5	2.024	105.6	2.007	101.6	2.124	132.9	2.095	124.4	2.146	139.8	2.054	113.2
Mean*	1.962	91.63	1.944	87.85	1.967	92.69	1.903	80.05	2.025	105.9	1.875	74.99
STD	0.066		0.067		0.145		0.178		0.110		0.162	
CV(%)	3.358		3.440		7.394		9.365		5.437		8.615	

*Geometric mean for BMD and BMDL on original scale